Effect of Water and Soil Qualities on Phytoplankton Die-offs in Intensive Pacific White Shrimp *(Litopenaeus vannamei)* Cultured Ponds

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ABSTRACT

The study is focused on the effect of water and soil qualities on phytoplankton die-offs in intensive Pacific white shrimp cultured ponds. Six earthen ponds (8,000 m^2/ pond) were used for rearing *Litopenaeus vannamei* at the stocking density of 113 PL/m^2. The experimental ponds were divided into two groups. First group consisted of three ponds which had unstable phytoplankton bloom from previous crop. Second group consisted of three ponds which had more stable phytoplankton community from previous crop. The water color shades were recorded throughout the culture period. Phytoplankton and water quality samples were collected every 7-10 days while soil samples were collected at feeding area every month until shrimp were harvested. Water coloration with light to light green were observed during water preparation before stocking postlarval shrimp and gradually changed to yellowish green and greenish brown after stocking. Greenish brown and yellowish green shades were the most common color shades observed during the culture period. After phytoplankton die-offs the coloration changed to dark green. Brown to dark brown color was observed prior to harvesting. Cyanophyceae, Chlorophyceae, Chromophyceae and Dinophyceae were the common groups of phytoplankton found in all experimental ponds. Average number of phytoplankton from six experimental ponds ranged between $5.5 \pm 6.4 \times 10^4$ to $2.2 \pm 2.8 \times 10^5$unit cell/land related with pH, DO and total phosphorus in the pond water but not correlated with soil quality. Statistical analysis compared between stable color ponds and unstable color ponds showed that the density of phytoplankton in the unstable water color ponds ($9.2 \pm 5.9 \times 10^4$ unit cell/l) was significantly lower than the stable water color ponds ($1.6 \pm 1.0 \times 10^5$ unit cell/l). Water pH and total phosphorus were significantly different between the groups ($P<0.05$). Unstable water color ponds had pH of 7.9±0.1 lower than the stable water color ponds of 8.0±0.1. Total phosphorus in unstable water color ponds (0.7±0.4 mg/l) was higher than the stable water color ponds (0.4±0.2 mg/l). Furthermore, total phosphorus was higher than 0.5 mg/l significantly decreased phytoplankton number. Water pH and total organic matter in soils were significantly different between treatments ($P<0.05$). The soil pH from unstable water color ponds (6.6±0.1) was lower than stable water color ponds (7.7±0.1) while an average

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of total organic matter from unstable water color ponds (2.8±0.87%) was higher than the stable water color ponds (2.2±0.59%). The average production, body weight and growth rate from stable color ponds were significantly different than the unstable water color ponds. However, survival rate and FCR from both groups were not significantly different.

Keywords: *Litopenaeus vannamei*, Phytoplankton community, Phosphorus, Water quality

INTRODUCTION

Since the decline of black tiger shrimp *(Penaeus monodon)* culture between 2001-2003, Thai shrimp farmers have switched to the Pacific white shrimp *(Litopenaeus vannamei)* culture. Currently, *L.vannamei* is rapidly replacing the black tiger shrimp as the major farmed species. The main reason for this change is that *L. vannamei* has faster growth rate, can be stocked at a higher stocking density, gives a higher yield and has a lower production cost than *P. monodon* (Limsuwan and Chanratchakool, 2004).

In intensive shrimp farming, phytoplankton management is becoming more and more important because low production and mortalities usually occur in ponds with an unstable phytoplankton bloom. Shrimp usually become sick and eventually die after a couple of consecutive massive phytoplankton die-offs in the ponds (Limsuwan, 2000). Phytoplankton die-offs lead to the formation of foam on the water surface and accumulation of dead phytoplankton on the pond bottom, resulting in poor water quality and deteriorating pond conditions (Chanratchakool *et al*., 1993). Subsequently, there is more stress on the shrimp, decreasing their performance (poor growth rate and feed conversion efficiency) and increasing their susceptibility to disease leading to mortality (Boyd and Musig, 1992; Wangwibulkit, 2008). Several researchers reported that phytoplankton die-off in intensive shrimp ponds in Thailand usually occurred due to the following: a) heavy phytoplankton bloom before die-off; b) lack of nutrients such as nitrogen and phosphorus in organic and inorganic forms; c) lack of sufficient sunlight during rainy or cloudy days; and, d) after a high rate of water exchange (Chanratchakool *et al*., 1993; Limsuwan, 2000; Limsuwan and Chanratchakool, 2004).

Most experienced shrimp farmers could differentiate the changes in phytoplankton bloom in the water by visual observation, i.e. variation in water color in different shades such as green, blue green, yellowish green, brown and reddish brown (Limsuwan, 2000). However, even in the same farm, the occurrence of phytoplankton crash among nearby ponds is extremely variable. The aim of this study was to determine the key essential factors (water and soil qualities) that correlated with phytoplankton bloom, and its eventual crash in intensive *L.vannamei* ponds.

MATERIALS AND METHODS

This study was carried out in an intensive shrimp farm located in Prachuapkhirikhan province, Southern Thailand, between March and July 2008. Six earthen ponds, with an area of 8,000 m^2/pond, were selected and divided into two groups for the experiment. The first group consisted of three ponds which had unstable phytoplankton bloom from the previous crop. The second group consisted of three ponds which had a more stable phytoplankton community from the previous crop. Pond preparation started from removing the sludge from the pond bottom after the shrimp were harvested. Bottom soil was ploughed and dried for 3 weeks before adding water from the reservoir until the level of 1.8 m was reached. Fertilizers, minerals and lime were applied to the pond water in order to stimulate natural food until Secchi disc visibility was 50-60 cm before stocking the postlarvae.

Postlarval stage 12 (PL 12) of *L.vannamei* were checked by polymerase chain reaction (PCR) assays to ensure they were free from white spot syndrome virus (WSSV), Taura syndrome virus (TSV) and yellow-head virus (YHV) before stocking at the rate of 113 PL/m^2/pond. Shrimp were fed with commercial pelleted feed 4 times a day (07:00, 12:00, 18:00 and 24:00) throughout the experiment. After 60 days post-stocking, the water in each pond was exchanged to maintain optimum water quality for shrimp grow the specially one month before harvesting. The shrimps were sampled to determine their average weight every 7 to 10 days from day 30 until harvest.

Phytoplankton Sampling and Analysis

The water color was observed by visual estimation during water preparation and after stocking the postlarvae. The color shades were recorded by visual estimation throughout the culture period until shrimp were harvested. For the phytoplankton study,10 liters of water sample was collected from each experimental pond at 30 cm depth for phytoplankton identification and density analysis every 7 to 10 days. The water samples were filtered through a 20 micrometer plankton net. Phytoplankton samples were kept in 135 ml plastic bottles and preserved in 4 % formalin. Phytoplankton numbers were enumerated by using the Sedgwick-Rafter counting chamber under a compound microscope following Wongrat and Boonyaphiwat (2003). Phytoplankton identification was carried out following the method of Wongrat (1998; 1999).

Water Sampling and Analysis

Water quality parameters were measured every 7 to 10 days. Water temperature, dissolved oxygen (DO), pH and salinity were measured by YSI DO 200-4M while transparency was measured by Secchi disc. The water samples were collected from 30 cm. depth and kept in 500 ml plastic bottles and preserved at 4ºC for water quality analysis at Aquaculture Business Research Center (ABRC) laboratory, Kasetsart University. The water quality parameters including hardness, total alkalinity, total ammonia-nitrogen, nitrite-nitrogen, total suspension solid (TSS) and chlorophyll *a* were analysed according to Strickland and Pearsons (1972). Total phosphorus was analyzed by standard method (APHA *et al*., 1995).

Soil Sampling and Analysis

Soil samples were collected from 5 cm depth at the feeding area once a month from each pond and kept in plastic bags. The soil samples were air dried and analyzed for pH, total organic matter by wet oxidation method (Allison, 1965), total nitrogen by semi-micro Kjeldahl method (Bremner and Mulvaney, 1982) and total phosphorus by $HClO_4$ and spectrophotometer (Olsen and Sommer,1982)

Data Analysis

The relationships between the density of phytoplankton, water and soil qualities during the culture period were analyzed by Pearson correlation method. At the end of the culture period, the density of phytoplankton, water and soil qualities, body weight, growth rate, survival rate and FCR were statistically compared using independent sample T-test method between groups (Steel and Torrie, 1980)

RESULTS

Water Color

Water color of the experimental ponds during pond water preparation before stocking was light green to green. After stocking, the color gradually changed to yellowish green and greenish brown. During the culture period the most common color shades of the pond water was greenish brown and yellowish green. However, after phytoplankton die-offs the coloration changed to dark green. Brown to dark brown color was observed prior to harvesting.

Phytoplankton,Water and Soil Qualities

There were 5 genera of Division Cyanophyceae (blue-green algae), 3 genera of Division Chlorophyceae, 2 genera and unidentified group of flagellate in Division Euglenophyceae (euglenoids), 6 genera of pennate diatom in Division Chromophyceae (diatom) and 2 genera of Division Dinophyceae (dinoflagellate). The dominant genera such as *Cyclotella* (Choromophyceae) and *Oscillatoria* (Cyanophyceae) fluctuated throughout the culture period. Average number of phytoplankton from the six experimental pondsranged between 5.5 $\pm 6.4\times10^4$ to 2.2 $\pm 2.8 \times10^5$cells/l and the density varied among the ponds. Water and soil quality parameters during the grow-out period were suitable for shrimp culture (Table 1 and 2). The phytoplankton abundance correlated with pH, DO and total phosphorus in the pond water (Table 3) but not with soil quality (Table 4).

Table 1. Quantity of phytoplankton and concentration of water quality parameters in the experimental ponds

Water quality parameter	Range	Mean ±s.d.
pH	7.2-1.02	8.07±0.15
Temperature (° C)	28.8-30.1	30±0.14
Salinity (psu)	33.9-35.0	34.5±0.55
DO (mg/l)	6.0-6.3	6.18±0.13
Transparency (cm.)	42.9-50.6	46.50±3.56
Total alkalinity (mg/l)	134.5- 138.84	137.67±10.17
Total hardness (mg/l)	6,684-6,888	6,786±102
Total ammonia (mg/l)	0.08-0.14	0.11±0.03
Nitrite-nitrogen (mg/l)	0.04-0.08	0.06±0.02
Nitrate-nitrogen (mg/l)	0.53-0.65	0.59±0.06
Total phosphorus (mg/l)	0.88-1.12	1.05±0.17
Total suspension solid (mg/l)	50.8-72.0	61.41±10.60
Chlorophyll *a* (mg/l)	39.6-45.68	42.67±3.01
Phytoplankton (cells/l)	$5.5 \pm 6.4 \times 10^4$-$2.2 \pm 2.8 \times 10^5$	$1.2 \pm 6.5 \times 10^5$

Table 2. Concentration of soil quality parameters in the experimental ponds

Soil quality parameter	Range	Mean ±s.d.
Soil pH	6.5-8.9	7.7+1.12
Total nitrogen (%)	0.2-0.38	0.29±0.09
Total phosphorus (mg/l)	0.22-0.32	0.27±0.05
Total organic matter (%)	2.17-2.89	2.53±0.36

Table 3. The correlation between phytoplankton and water quality

Water quality	plankton	Water quality	plankton
pH	0.251*	Total hardness (mg/l)	-0.27
Temperature (° C)	0.15	Total ammonia (mg/l)	-0.04
Salinity (psu)	-0.01	Nitrite-nitrogen (mg/l)	-0.08
DO (mg/l)	0.219*	Nitrate-nitrogen (mg/l)	-0.03
EC (mS/cm)	0.01	Total phosphorus (mg/l)	-0.201*
Transparency (cm)	0.10	Total suspension solid (mg/l)	0.01
Total alkalinity (mg/l)	-0.06	Chlorophyll *a* (mg/l)	-0.06

Note: *showed significant correlation (P<0.05)

Table 4. The correlation between phytoplankton and soil quality

	Soil quality		
	Total nitrogen	Total phosphorus	Total organic matter
Phytoplankton	-0.192	-0.181	0.119

Note:*showed significantly correlation (P<0.05)

Moreover, in this study,fluctuation of phytoplankton was observed during culture period in the more stable phytoplankton ponds. The average phytoplankton density in the ponds with unstable colored was 9.2 $\pm 5.9\times10^4$ cells/l which was significantly lower than that in the more stable color ponds ($1.6\pm1.0\times10^5$ cells/l (P<0.05) (Table 5). The average concentrations of water quality parameters such as temperature, salinity, DO, transparency, total alkalinity, total hardness, total ammonia, nitrite-nitrogen, TSS and chlorophyll *a* between unstable and stable water color ponds were not significantly different (P>0.05). However, average pH and total phosphorus levels were significantly different between the groups (P<0.05). The average pH in the unstable water color ponds was 7.9±0.1, while that of the more stable water color ponds was 8.0±0.1. The average total phosphorus concentration in the unstable water color ponds was 0.7±0.4 mg/l which was significantly higher than that in ponds with more stable water color which had 0.4 ±0.2mg/l.

Table 5. Average number of phytoplankton (cells/l) and water quality condition between unstable and stable water color ponds

	unstable water color ponds	**stable water color ponds.**
Phytoplankton (cells/l)	$9.2\pm5.9\times10^{4a}$	$1.6\pm1.0\times10^{5b}$
pH	7.9 ± 0.1^{a}	8.0 ± 0.1^{b}
Temperature (° C)	29.6 ± 1^{a}	29.7 ± 0.9^{a}
Salinity (psu)	31.8 ± 2.3^{a}	31.6 ± 1.5^{a}
DO (mg/l)	5.6 ± 2.2^{a}	5.6 ± 2.4^{a}
Transparency (cm)	21 ± 8.3^{a}	25 ± 10^{a}
Total alkalinity (mg/l)	132 ± 22^{a}	135 ± 25^{a}
Total hardness (mg/l)	$6,435\pm230^{a}$	$6,344\pm23^{a}$
Total ammonia (mg/l)	1.8 ± 1.2^{a}	2.3 ± 1.8^{a}
Nitrite-nitrogen (mg/l)	1.0 ± 1.1^{a}	0.94 ± 0.8^{a}
Nitrate-nitrogen (mg/l)	0.30 ± 0.1^{a}	0.42 ± 0.2^{a}
Total phosphorus (mg/l)	0.7 ± 0.4^{a}	0.4 ± 0.2^{b}
Total suspension solid (mg/l)	$1,318\pm1,793^{a}$	$2,418\pm4,193^{a}$
Chlorophyll *a* (mg/l)	140 ± 125^{a}	142 ± 147^{a}

Note: differentalphabets in the same row show significant differences (P<0.05)

The concentrations of soil quality parameters between experimental ponds are shown in Table 6. Total nitrogen and total phosphorus did not differ between two treatments (P>0.05) whilst pH and total organic matter were significantly different between the treatments (P<0.05). The average soil pH from the ponds with unstable water color was 6.6±0.1 which is significantly lower than that of the stable water color ponds (7.7±0.1), while total organic matter in the unstable water color ponds (2.8±0.87 %) was higher than that in the more stable water color ponds (2.2±0.59 %). Furthermore, phytoplankton density was related to total phosphorus in the water column. When total phosphorus was higher than 0.5 mg/l, a significant decrease in phytoplankton number was observed. In the unstable water color ponds, total phosphorus in the water tended to increase as the culture period extended. The abundance of phytoplankton was related to total phosphorus in pond water, however, when the total phosphorus level was higher than 0.5 mg/l, phytoplankton die-offs were frequently observed (Figure 1).

Table 6. Soil quality conditions between unstable and stable water color ponds

	unstable water color ponds	**stable water color ponds.**
pH	6.6 ± 0.1^{a}	7.7 ± 0.1^{b}
Total nitrogen (%)	0.29 ± 0.13^{a}	0.28 ± 0.09^{a}
Total phosphorus (mg/l)	0.24 ± 0.12^{a}	0.28 ± 0.09^{a}
Total organic matter (%)	2.8 ± 0.87^{a}	2.2 ± 0.59^{b}

Note: different alphabets in the same row show significant differences (P<0.05)

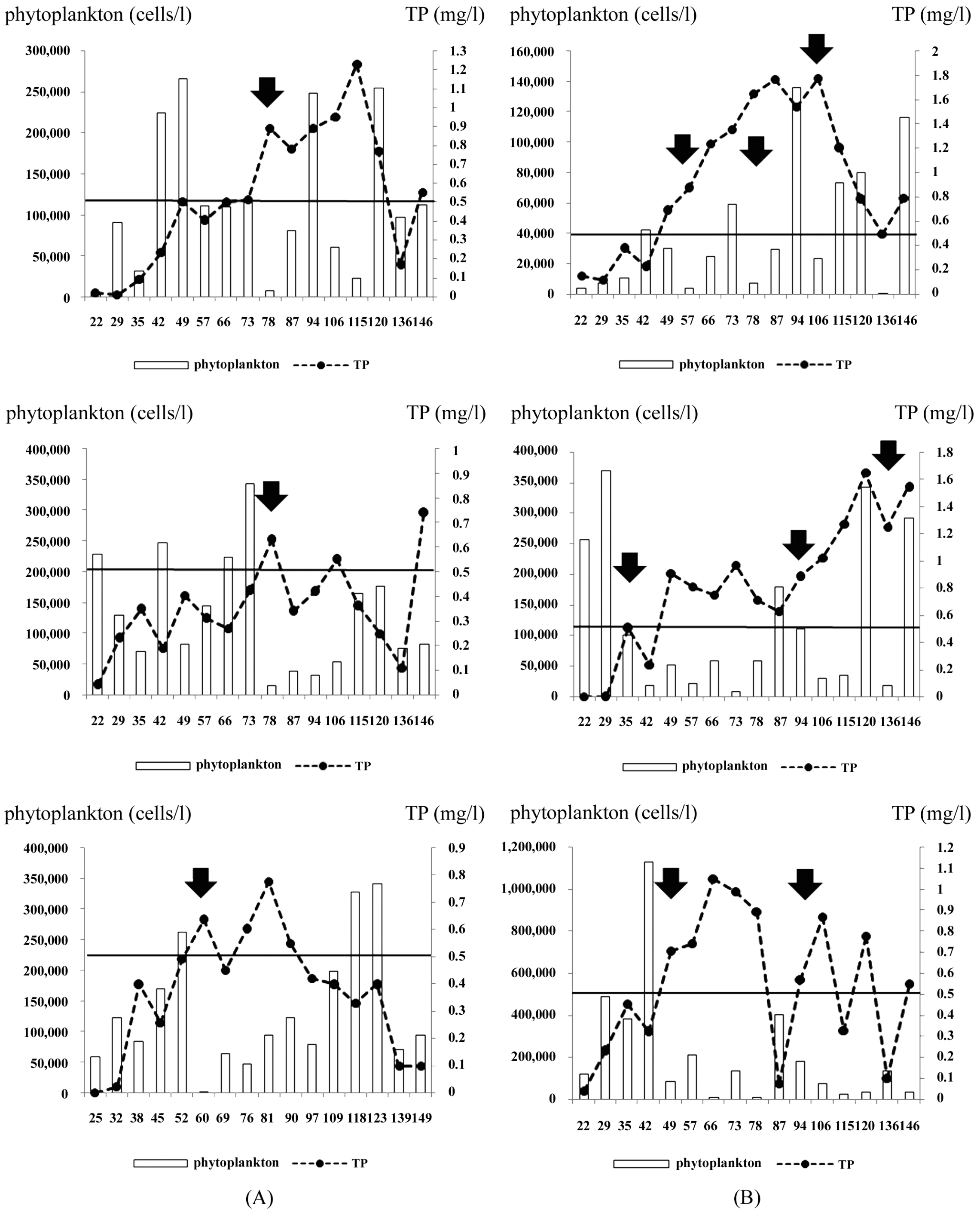

Figure 1. Correlation between phytoplankton and total phosphorus in water.
A: stable water color ponds;
B: unstable water color ponds.
(The arrow points to phytoplankton crash when total phosphorus is more than 0.5 mg/l)

The production of shrimp, body weight, growth rate, survival rate and FCR from unstable and stable water color ponds are shown in Table 7. The average production, body weight and growth rate from stable color ponds were significantly different from those in the unstable water color ponds. However, survival rate and FCR from both groups were not significantly different.

Table 7. Production, weight gain, growth rate, survival rate and FCR between unstable and stable water color ponds

Experimental pond	production (kg/rai)	body weight (g/ind.)	Growth rate (g/day)	Survival rate (%)	FCR
unstable water color ponds	1,443±900 [a]	13.8±3.32 [a]	0.12±0.13 [a]	54.7±0.6 [a]	1.61±0.55 [a]
stable water color ponds	1,884±955[b]	15.22±4.22[b]	0.18±0.52[b]	61.6±7.8 [a]	1.58±0.21 [a]

Note: Different alphabets in the same column show significant differences ($P<0.05$)

DISCUSSION

Phytoplankton abundance in the shrimp ponds correlated with pH, DO and total phosphorus in the water. Boyd and Tucker (1998) reported that the exchange of phosphorus between water column and bottom soil made phosphorus the limiting factor for phytoplankton growth. The solubility of phosphorus inorganic compounds (aluminium, calcium and iron) was dependent on the pH of the mud. Busman *et al*. (2009) reported that phosphorus availability related with all ranges of soil pH but could be pronounced in ponds with fluctuating water color. The average soil pH was 6.6±0.1 which was within the pH range that would allow phosphorus from the pond bottom to be released to the pond water, there by stimulating phytoplankton growth (Figure 2). Thus, a pH of less than 6.5 would allow total phosphorus in the bottom soil to be dissolved into the water column much more than at higher pH levels. Total phosphorus levels were higher in the unstable water color ponds than in the stable water color ponds. The results from this study showed that phytoplankton die-offs due to high concentrations of phosphorus stimulated quick phytoplankton growth resulting in rapid die-offs. Boyd and Tucker (1998) suggested that the optimum concentration ranges of total phosphorus should be between 0.005-0.2 mg/l.

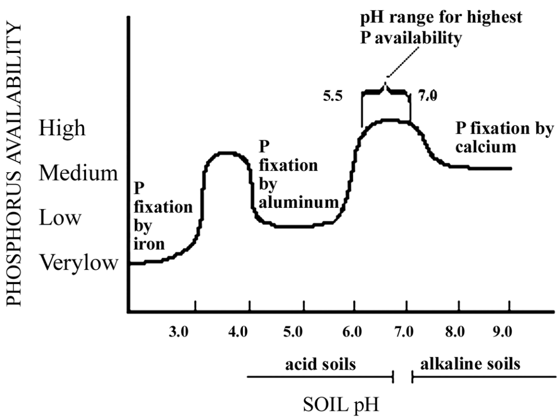

Figure 2. The availability of phosphorus is affected by soil pH. (Source: Busman *et al*., 2009)

The major source of phosphorus in shrimp ponds is pelleted feed (Funge-Smith and Briggs, 1998). Phosphorus is absorbed by plants and mud rapidly (Boyd and Tucker, 1998). Seo and Boyd (2001) suggested that drying and ploughing the pond bottom after harvesting will solve this situation. Ploughing the bottom soil will expose soil surface to water, allow soil to absorb phosphorus, and reduce phosphorus release from an aerobic condition. In the stable color ponds, the average soil pH was 7.7±0.1, a condition which allows the phosphorus in the pond bottom to be fixed by calcium. When phytoplankton blooms, some shrimp farmers use biocides such as Benzalkonium Chloride (BKC), formalin, glutaraldehyde and calcium hypochlorite to control phytoplankton density fromover-blooming (Funge-Smith and Briggs, 1998) or exchange water to remove excess plankton and nutrients from the pond to control phytoplankton bloom and maintain optimum water quality in shrimp ponds (Funge-Smith, 1996). In this study, in order to control the abundance of phytoplankton die-offs during the grow-out period, phosphorus in the pond water and bottom soils should be controlled by ploughing and drying the pond after harvesting the shrimp. Proper feeding management during culture period should be done to ensure that the suitable level of phosphorus and other nutrients accumulated in the ponds.

CONCLUSION

The present study indicated that phytoplankton die-offs are related to total phosphorus concentration in the water column. A total phosphorus concentration of more than 0.5 mg/l affects phytoplankton density and leads to phytoplankton die-offs. The solubility of phosphorus is affected by soil pH in the 5.5-7.0 range. Therefore pond preparation activities such as drying and ploughing the bottom soil after harvesting can remove phosphorus from the pond bottom. This is the method of choice to eliminate excessive phosphorus in the soil.

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LITERATURE CITED

Allison, L.F. 1965. Organic carbon, pp 1,372 -1,375. *In* C.A. Black, ed. **Method of Soil Analysis. Part 2.** Agronomy No.9 Am.Soc.Agron. Madison, Wisconsin. USA.

APHA, AWWA. and WEFA. 1995. **Standard Method for the Examination of Water and Wastewater.** 20th edition. United Book Press, Maryland.

Boyd, C.E. and C.S. Tucker. 1998. **Pond Aquaculture Water Quality Management.** Alabama Agricultural Experimental Station, Auburn University, Alabama. 183 p.

Boyd, C.E. and Y.Musig. 1992. Shrimp pond effluents: observations of the nature of the problem on commercial farms,pp 195-197. *In* : J.Wyban, ed. **Proceeding of the Special Session on Shrimp Farming.** World Aquaculture Society, Baton Rouge

Boyd, C.E. and D. Gautier. 2000. Effluent composition and water quality standard. **Global Aquaculture Advocate.** 3(5): 61-66.

Bremner, J.M. and C.S. Mulvaney. 1982. Nitrogen-Total, pp 595-624. *In* A.L. Page, R.H. Miller and D.R. Keeney, eds. **Methods of Soil Analysis,** Part 2, Agronomy No.9, Madison, Wisconsin, USA.

Busman,L., J. Lamb, G. Randall, G. Rehm and M. Schmitt. 2009. **The Nature of Phosphorus in Soil.** Regents of the University of Minnesota, USA.

Chanratchakool, P., J.F. Turnbull and C. Limsuwan. 1993. **Health Management in Shrimp Ponds. Aquatic Animal Health Research Institute.** Kasetsart University Campus, Bangkok. 91 p.

Funge-Smith, S.J., 1996. Water and sediment quality in different intensive shrimp culture systems in southern Thailand. *In*: **Coastal Aquaculture and Environment: Strategies for Sustainability.** ODA Research Project R6011, Institute of Aquaculture, University of Stirling, Scotland.

Funge-Smith, S.J. and M.R.P. Briggs. 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. **Aquaculture.**164:117-133.

Limsuwan, C. 2000. **Thai Shrimp 2000.** Jaroenrat Printing, Bangkok. 260 p. (in Thai)

Limsuwan, C. and P. Chanratchakool. 2004. **Shrimp Industry of Thailand,** Magic Publication Co, Ltd. Bangkok. 206 p. (in Thai)

Olsen, S.R., and L.E. Sommer.1982. Phosphorus, pp 403-430. *In* A.L. Page *et al*., eds. **Method of Soil Analysis.** Part 2. Am. Soc.of Agron, Inc. Madison,Wisconsin. U.S.A.

Steel, R.G.D. and J.H. Torrie.1980. **Principles and Procedures of Statistics: A Biometrical Approach.** 2nd ed. McGraw -Hill Inc., New York.

Strickland, J.D.H. and T.R. Parsons. 1972. **A Practical Handbook of Seawater Analysis.** 2nd ed. Fisheries Research Board of Canada, Bulletin 169, Ottawa, Canada.

Wangwibulkit, S., C. Limsuwan and N. Chuchird. 2008. Effect of salinity and pH on the growth of blue-green algae, *Oscillatoria* sp. and *Microcystis* sp., isolated from Pacific white shrimp (*Litopenaeus vannamei*) Ponds.**KU. Fish. Res. Bull.** 32(1): 1-9.

Wongrat, L. 1998. **Zooplankton**. Kasetsart University Press, Bangkok. 787 p. (in Thai)

Wongrat, L. 1999. **Phytoplankton.** Kasetsart University Press, Bangkok. 851 p. (in Thai)

Wongrat, L. and S. Boonyaphiwat. 2003. **Manual of Sampling and Analytical Methods of Plankton.** Kasetsart University Press, Bangkok. 270 p. (in Thai)

Seo, J. and C.E. Boyd. 2001. Effect of bottom soil management practices on water quality improvement in channel catfish *Ictaluruspunctatus* ponds. **Aquaculture Engineering.** 25:83-97.